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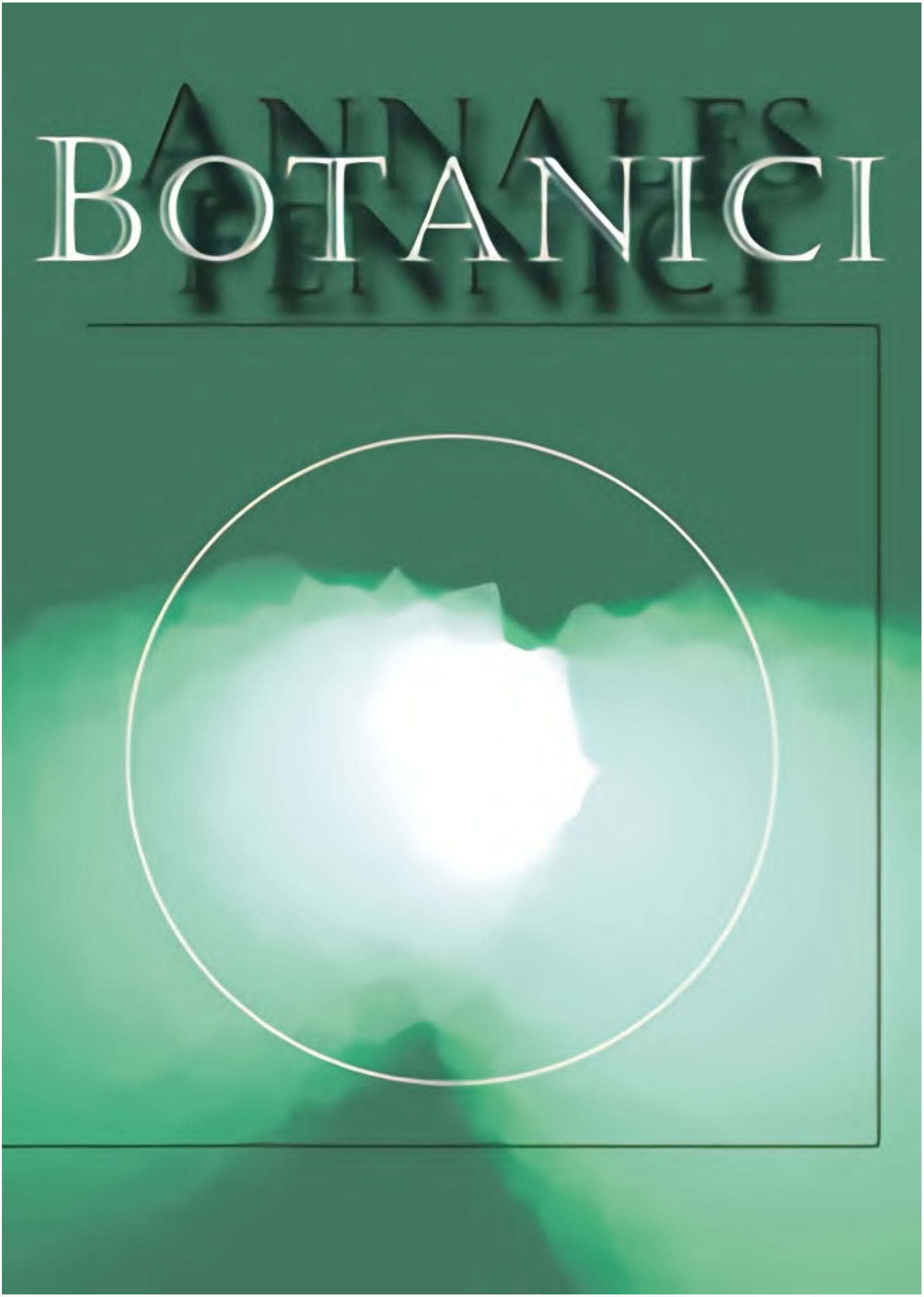

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Hyphodermella aurantiaca sp. nova (Polyporales, Basidiomycota) as evidenced by morphological characters and phylogenetic analyses

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Hyphodermella aurantiaca C.L. Zhao *sp. nova* (Polyporales, Basidiomycota) is described based on morphological and molecular evidence. The species has an annual growth habit, resupinate basidiomata with saffron to orange hymenial surface, a monomitic hyphal system with generative hyphae bearing simple septa, IKI–, CB–, and ellipsoid, hyaline, thin-walled, smooth, IKI–, CB– basidiospores. In phylogenetic analyses based on molecular data of ITS + nLSU and nLSU sequences *H. aurantiaca* formed a monophyletic lineage with a strong support (100% BS, 100% BP, 1.00 BPP) and grouped with *H. corrugata*, *H. poroides* and *H. rosae*.

Introduction

Hyphodermella was typified by *H. corrugata* (Eriksson & Ryvarden 1976). The genus is characterized by resupinate, crustose basidiomata, smooth to grandinioid to odontioid or hydnoid or poroid hymenophore and a monomitic hyphal structure with simple septa on generative hyphae, presence of encrusted hyphal ends, clavate to suburniform basidia, and ellipsoid to globose, smooth, thin-walled basidiospores (Eriksson & Ryvarden 1976, Bernicchia & Gorjón 2010, Duhem & Buyck 2011). So far seven species have been accepted in the genus worldwide (Gilbertson *et al.* 2001, Hjortstam & Ryvarden 2007, Ryvarden 2007, Nakasone 2008, Duhem 2010).

Recently, molecular studies on *Hyphodermella* based on single-gene or multi-gene data sets were carried out and the type species or related species of *Hyphodermella* were placed in Phanerochaetaceae (Larsson 2007, Binder *et al.* 2013, Floudas & Hibbett 2015, Justo *et al.* 2017, Zhao *et al.* 2017). Larsson (2007) presented a classification of corticioid fungi based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences and showed that *H. corrugata* grouped with *Phlebia firma* in the Phanerochaetaceae. Binder *et al.* (2013) presented molecular studies employing multi-gene — 5.8S, nLSU, translation elongation factor 1-α (TEF1) gene, mitochondrial rRNA gene sequences (mtSSU),

the second-largest subunit of RNA polymerase II (RPB2) and the largest subunit of RNA polymerase II — data sets to investigate the phylogenetic relationships within the Polyporales, in which *H. corrugata* was nested in the phlebioid clade as a lineage diverging just before *Terana coerulea*. The genus *Phanerochaete* was analysed using a four-gene data set; in that analysis *H. rosae* grouped with *Pirex concentricus* in the phlebioid clade (Floudas & Hibbett 2015). In a revised family-level classification of the Polyporales *H. rosae* clustered with *Donkia pulcherrima* and *Pirex concentricus* (Justo *et al.* 2017). Zhao *et al.* (2017) introduced a new *Hyphodermella* species, *H. poroides*, based on morphological and molecular evidence, and in their analysis *H. corrugata*, *H. poroides* and *H. rosae* grouped together.

During investigations on wood-inhabiting fungi in southern China, we found polypores which could not be assigned to any described species. Here, we used sequences from previous studies to examine the taxonomy and phylogeny of this new species within *Hyphodermella*, based on the internal transcribed spacer (ITS) regions and the large subunit nuclear ribosomal RNA gene (nLSU) sequences.

Material and methods

Morphological studies

The specimens studied are deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macro-morphological descriptions are based on field notes. The colour terms follow Petersen (1996). Micro-morphological data were obtained from dried specimens, and observed under a light microscope following Dai (2012). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and indextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), $Q = L/W$ ratio, n (a/b) = number of spores (a) measured from given number (b) of specimens.

Molecular techniques and phylogenetic analyses

CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing) was used to obtain genomic DNA from dried specimens, according to the manufacturer's instructions. A small piece of dried fungal specimen (about 30 mg) was ground to powder in liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer, and incubated in a 65 °C water bath for 60 min. After that, 0.4 ml phenol–chloroform (24:1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13 000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13 000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added into the AC for a centrifugation at 12 000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube, and 100 ml elution buffer was added to the middle of adsorbed film to elute the genome DNA. The ITS region was amplified with primer pairs ITS5 and ITS4 (White *et al.* 1990). The nuclear LSU region was amplified with primer pairs LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

Sequences were aligned in MAFFT 6 (Katoh & Toh 2008; https://mafft.cbrc.jp/alignment/server/index.html) using the G-INS-I and E-INS-I strategies for nLSU and ITS + nLSU, respectively, and manually adjusted in BioEdit (Hall 1999). The alignment data sets were deposited in

TreeBase (submission ID 25004). A sequence for *Candelabrochaete africana* obtained from GenBank was used as an outgroup to root the nLSU phylogenetic tree following Justo *et al*. (2017), and *Pirex concentricus* was selected as an outgroup for phylogenetic analyses of ITS + nLSU region following Zhao *et al*. (2017).

Maximum parsimony analyses were applied to the ITS + nLSU and nLSU data set sequences. Approaches to phylogenetic analysis followed Chen *et al.* (2016), and the tree construction procedure was performed in PAUP* ver. 4.0b10 (Swofford 2002). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree generated. Sequences were also analysed using Maximum Likelihood (ML) with RAxML-HPC2 in the Cipres Science Gate-

Table 1. List of species, specimens, and GenBank accession number of sequences used in this study.

Species	Specimen	GenBank accession no.	
		ITS	nLSU
Bjerkandera adusta	*CBS 371.52*	–	MH868620
B. adusta	*HHB 12826*	–	KP135198
Candelabrochaete africana	*FP 102987*	–	KP135199
Hapalopilus rutilans	*FO 29328*	–	AF291333
H. rutilans	*CBS 422.48*	–	MH867966
Hyphodermella aurantiaca	*C.L. Zhao 10480*	MW209022	MW209011
H. aurantiaca	*C.L. Zhao 10487*	MW209023	MW209012
H. aurantiaca	*C.L. Zhao 10491*	MW209024	MW209013
H. aurantiaca	*C.L. Zhao 10500*	MW209025	MW209014
H. aurantiaca	*C.L. Zhao 10508*	MW209026	MW209015
H. aurantiaca	*C.L. Zhao 10510*	MW209027	MW209016
H. aurantiaca	*C.L. Zhao 10519*	MW209028	MW209017
H. aurantiaca	*C.L. Zhao 10521*	MW209029	MW209018
H. aurantiaca	*C.L. Zhao 10523*	MW209030	MW209019
H. aurantiaca	*C.L. Zhao 10525*	MW209031	–
H. aurantiaca	*C.L. Zhao 10530*	MW209032	MW209020
H. aurantiaca	*C.L. Zhao 10551*	MW209033	MW209021
H. corrugata	*MA-Fungi 26186*	FN600379	JN939585
H. corrugata	*MA-Fungi 24238*	FN600378	JN939586
H. poroides	*Dai 12045*	KX008367	KX011852
H. poroides	*Dai 10848*	KX008368	KX011853
H. rosae	*FP 150552*	KP134978	KP135223
H. rosae	*MA-Fungi 38071*	FN600389	JN939588
Phanerochaete ericina	*HHB 2288*	–	KP135247
P. laevis	*HHB 15519*	–	KP135249
P. magnoliae	*HHB 9829*	–	KP135237
P. pseudosanguinea	*FD 244*	–	KP135251
P. rhodella	*FD 18*	–	KP135258
Phlebiopsis gigantea	*FP 70857*	–	KP135272
Pirex concentricus	*OSC 41587*	KP134984	KP135275
Porostereum spadiceum	*CBS 476.48*	–	MH867985
Rhizochaete brunnea	*MR 229*	–	AY219389
R. fouquieriae	*KKN 121*	–	AY219390
Terana caerulea	*CBS 163.56*	–	MH869102
T. caerulea	*FP 104073*	–	KP135276

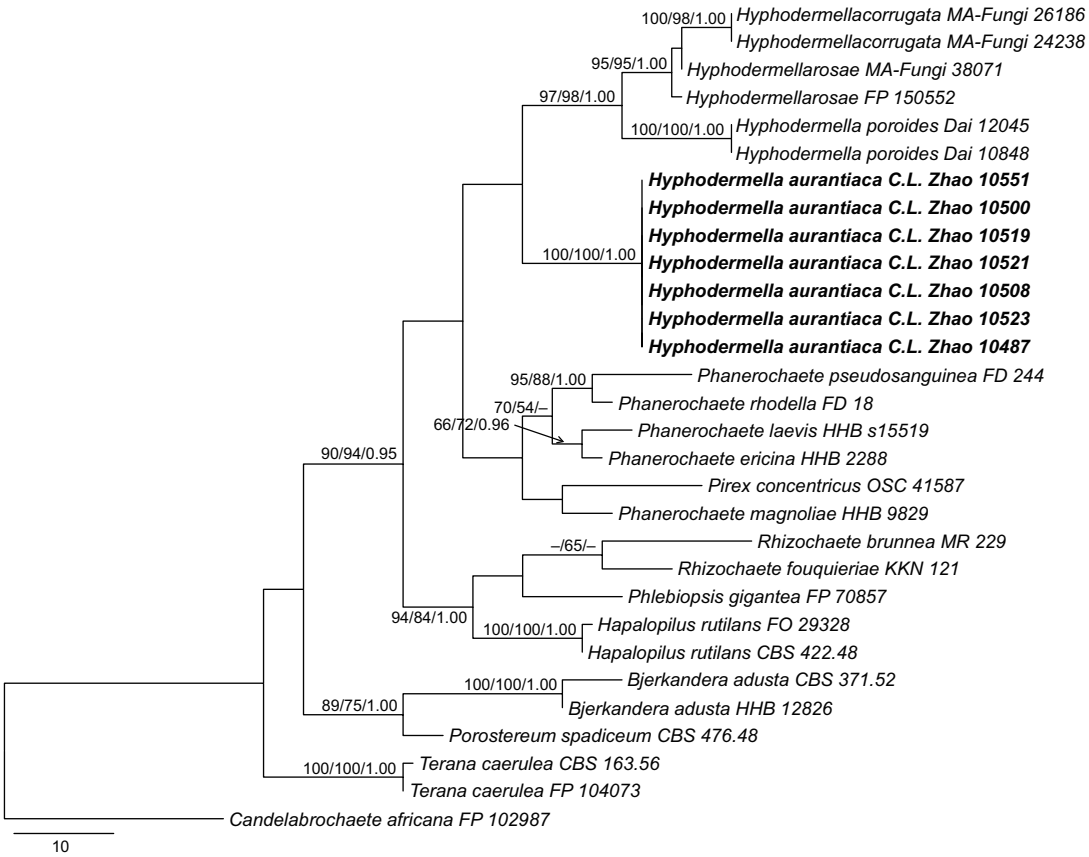

Fig. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Hyphodermella aurantiaca* and related species in *Hyphodermella* based on nLSU sequences. Branches are labelled with maximum likelihood bootstrap values higher than 70%, parsimony bootstrap values higher than 50%, and Bayesian posterior probabilities greater than 0.95, respectively.

way (http://www.phylo.org/sub_sections/portal). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes 3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 300 000 generations for nLSU and 3 million generations for ITS + nLSU, and trees were sampled every 100 generations. The first one-fourth of generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered significantly supported if they received maximum likelihood bootstrap value (ML) > 75%, maximum parsimony bootstrap value (MP) > 75%, or Bayesian posterior probabilities (PP) > 0.95.

Results

Molecular phylogeny

The nLSU data set (Fig. 1) included sequences from 30 fungal specimens representing 18 species. The aligned length of the data set was 1209 characters, of which 1048 were constant, 66 variable and parsimony-uninformative, and 95 parsimony-informative. Maximum parsimony analysis yielded three equally parsimonious trees (TL = 317, CI = 0.618, HI = 0.382,

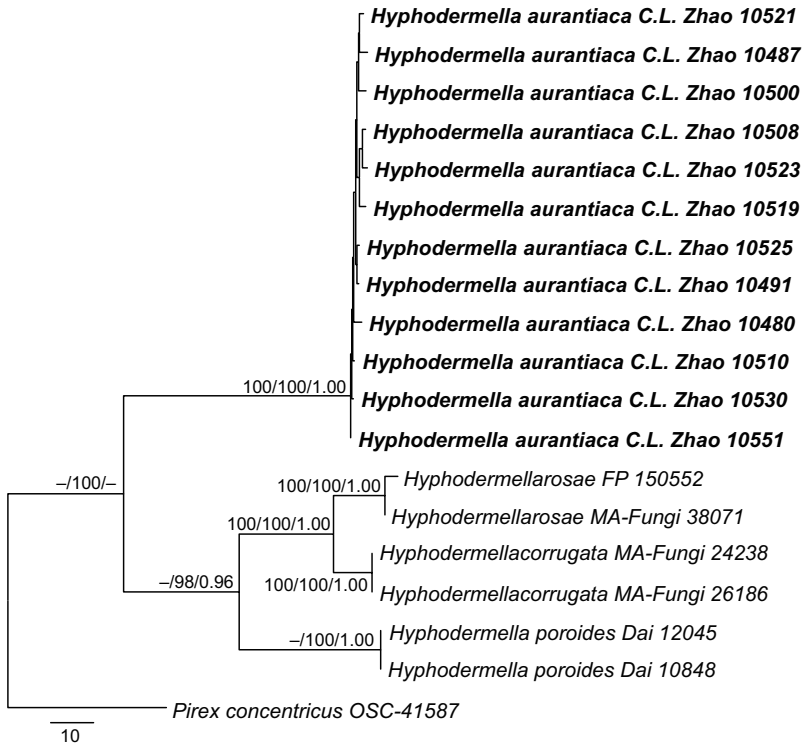

Fig. 2. Maximum parsimony strict consensus tree illustrating the phylogeny of 15 *Hyphodermella aurantiaca* and related species in *Hyphodermella* based on ITS + nLSU sequences. Branches are labelled with maximum likelihood bootstrap values higher than 70%, parsimony bootstrap values higher than 50%, and Bayesian posterior probabilities greater than 0.95, respectively.

RI = 0.751, RC = 0.464). The best model for the nLSU data set estimated and applied in the Bayesian analysis was GTR + I + G, *lset nst* = 6, rates = *invgamma*; *prset statefreqpr = dirichlet* (1,1,1,1). Bayesian and ML analyses produced similar topologies as MP analysis, with an average standard deviation of split frequencies = 0.009889 (BI).

In the phylogeny (Fig. 1) inferred from nLSU sequences, *H. aurantiaca* grouped with *H. corrugata*, *H. poroides* and *H. rosae* and then clustered with a clade comprising other genera as *Bjerkandera*, *Hapalopilus*, *Phanerochaete*, *Phaeophlebiopsis*, *Pirex*, *Rhizochaete* and *Terana*.

The ITS + nLSU data set (Fig. 2) included sequences from 19 fungi specimens representing five species. Its aligned length was 2079 characters, of which 1853 were constant, 57 variable and parsimony-uninformative, and 169 parsimony-informative. Maximum parsimony analysis yielded four equally parsimonious trees (TL = 370, CI = 0.760, HI = 0.241, RI = 0.843, RC = 0.641). The best model for the ITS + nLSU data set estimated and applied in the Bayesian analysis was GTR + I + G, *lset nst* = 6, rates = *invgamma*; *prset statefreqpr = dirichlet* (1,1,1,1). Bayesian and ML analyses produced similar topologies as MP analysis, with an average standard deviation of split frequencies = 0.009970 (BI).

In the phylogeny (Fig. 2) inferred from ITS + nLSU sequences obtained for related taxa of *Hyphodermella*, *H. aurantiaca* formed a monomitic lineage and grouped with *H. corrugata*, *H. poroides* and *H. rosae* with a strong support (100% BS, 100% BP, 1.00 BPP).

Taxonomy

Hyphodermella aurantiaca C.L. Zhao, *sp. nova* (Figs. 3 and 4)

MB 837949. — Holotype: China. Yunnan Province: Dali, Nanjian County, Lingbaoshan National Forestry Park, on fallen branch of angiosperm, 24°42´45.0´´N, 100°36´10.5´´E, 10 January 2019 *C.L. Zhao 10487* (SWFC0010487). rRNA gene sequence *ex* holotype: MW209023 (ITS), MW209012 (nLSU). — Paratypes: China. Yunnan Province: Dali, Nanjian County, Lingbaoshan National Forestry Park, on fallen branch of angiosperm, 24°42´22.1´´N, 100°36´10.3´´E, 10 January 2019 *C.L. Zhao 10480 (SWFC0010480)*, *C.L. Zhao 10491 (SWFC0010491)*, *C.L. Zhao 10500 (SWFC0010500)*,

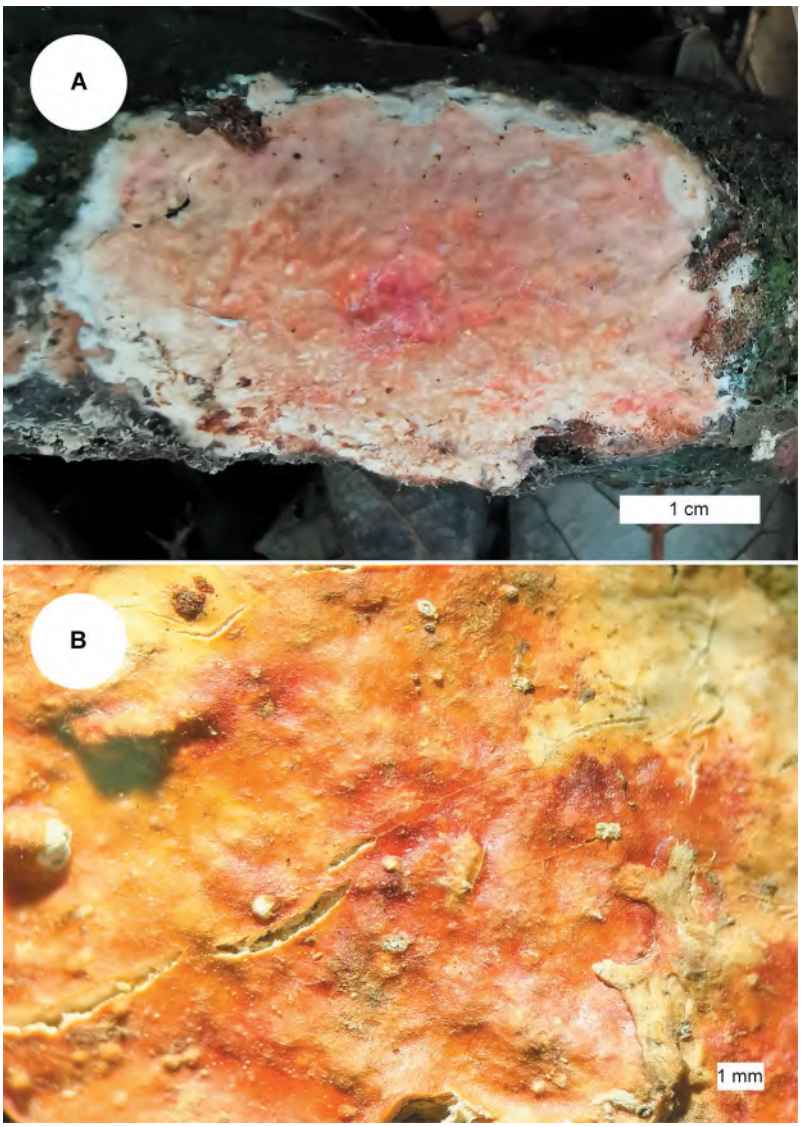

Fig. 3. Basidiomata of *Hyphodermella aurantiaca* (from the holotype). — **A**: Whole basidioma. — **B**: Close-up of the upper surface of basidioma.

C.L. Zhao 10508 (SWFC0010508), *C.L. Zhao 10510 (SWFC 0010510)*, *C.L. Zhao 10519 (SWFC0010519)*, *C.L. Zhao 10521 (SWFC0010521)*, *C.L. Zhao 10523 (SWFC0010523)*, *C.L. Zhao 10525 (SWFC0010525)*, *C.L. Zhao 10530 (SWFC 0010530)*, *C.L. Zhao 10551 (SWFC0010551)*.

ETYMOLOGY: The specific epithet *aurantiaca* (Lat.) refers to the species saffron to orange hymenial surface.

Basidiomata annual, resupinate, ceraceous when fresh, turn to leather upon drying, up to 12 cm long, 5 cm wide, 300–500 µm thick. Hymenial surfaces smooth to tuberculate, flesh to rose when fresh, saffron to orange upon drying. Sterile margin distinct, white. Hyphal structure monomitic; generative hyphae bearing simple septa, IKI–, CB–; tissues unchanged in KOH; subiculum absent or indistinct, hymenium thickening, hyphae colorless, more or less interwoven, thin-walled, branched, 2–4 µm in diameter; numerous crystals present among the abhymenium hyphae. Hymenium cystidia and cystidioles absent; basidia narrowly clavate, with four sterigmata and a basal simple septa, 10–20 × 2–4.5 µm; basidioles dominant, in shape similar to basidia, but slightly smaller. Spores ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (2.8)3–4(4.2) × 2–2.8 µm, L = 3.66 µm, W = 2.38 µm,

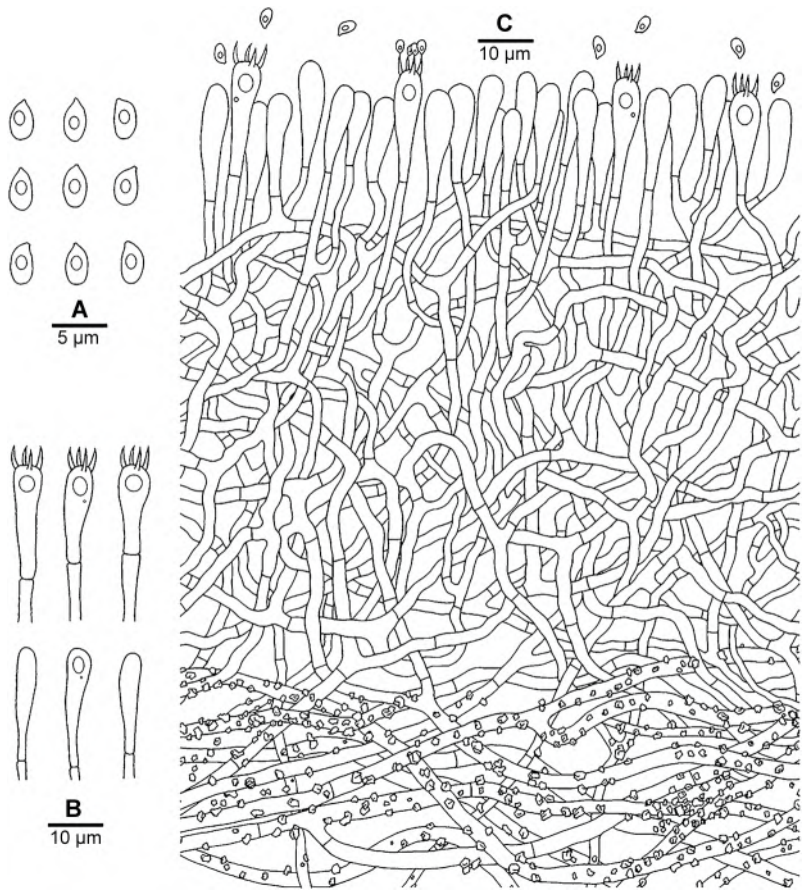

Fig. 4. Microscopic structures of *Hyphodermella poroides* (from the holotype). — **A**: Basidiospores. — **B**: Basidia and basidioles. — **C**: A section of hymenium.

Q = 1.48–1.62 (n = 360/12). Associated wood-rot, white.

Discussion

Based on the combined ITS + nLSU and nLSU sequence data, *H. aurantiaca* is closely related to *H. corrugata*, *H. poroides* and *H. rosae* (Fig. 1). However, morphologically, *H. corrugata* differs from *H. aurantiaca* by its grandinioid hymenophore and larger basidiospores (8–10 × 5–7 µm; Eriksson & Ryvarden 1976, Bernicchia & Gorjón 2010). *Hyphodermella poroides* can be distinguished from the new species by its poroid hymenophore and slightly cyanophilous walls on generative hyphae (Zhao *et al.* 2017).

Morphologically, *H. brunneocontexta*, *H. maunakeaensis*, *H. ochracea* and *H. rosae* resemble *H. aurantiaca* by having simple septa on generative hyphae and encrusted hyphal ends. However, *H. brunneocontexta* differs from *H. aurantiaca* by having an odontioid hymenophore, a brown subiculum and larger basidiospores (4.5–5 × 3.5–4 µm; Duhem & Buyck 2011), *H. maunakeaensis* by a hydnoid hymenophore and larger basidiospores (4.5–5 × 3.5–4 µm; Gilbertson *et al.* 2001), *H. ochracea* by its odontioid hymenophore, ochraceous subiculum and larger basidiospores (8–12 × 4–5.5 µm; Duhem 2010), and *H. rosae* by its odontioid hymenophore, and larger basidia (24–35 × 6–8 µm; Nakasone 2008).

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